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Zachary R. Donais

University of Connecticut - Storrs, zachary.donais@uconn.edu

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**A study of Native Parasitoids and Rearing Diet Efficacy for the Brown Marmorated
Stink Bug *Halyomorpha halys***

Zachary Richard Donais

B.S., University of Connecticut 2011

A Thesis

Submitted in partial fulfillment of the

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At the

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Approval Page

Masters of Science Thesis

**A study of Native Parasitoids and Rearing Diet Efficacy for the Brown
Marmorated Stink Bug *Halyomorpha halys***

Presented by Zachary Richard Donais, B.S.

Major Advisor_____

Ana Legrand

Associate Advisor_____

Richard McAvoy

Associate Advisor_____

Jane O'Donnell

University of Connecticut

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Abstract

The brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål), (Hemiptera; Pentatomidae) is an insect native to Asia. It was first discovered in the United States in the mid-1990s near Allentown, Pennsylvania. It was found in Connecticut in 2008 and since then numbers have continuously grown to where the pest is becoming an agricultural problem. A study was done to detect what native parasitoids attack the BMSB. Two different parasitoids were detected along with numerous accounts of egg predation. A second study focused on the effect of rearing diets on BMSB development. Time to maturation and longevity were significantly affected by diet type.

Thus, in order to determine the best rearing techniques, individual insects were taken from egg masses and put on seven different regularly available diet treatments and monitored daily. The diets with the best overall survival were carrot, bean carrot, and apple carrot. Apple carrot, carrot, apple, and bean carrot had the longest average survival time with detectable differences. On average, bean carrot, bean and autumn olive had the shortest periods between molts.

Native parasitoid populations were surveyed by using egg masses from three different stink bug species collected from lab-reared colonies, frozen at -17°C to render them unviable and placed into three different types of habitats, where they were later assessed for evidence of predator or parasitoid activity. Habitat types were a corn field, an ornamental landscape, and natural areas. Initial tests showed that natural areas had the most parasitoids detected, while ornamental plant areas showed the greatest amount of predator damage to egg masses. Two parasitoids were detected as parasitizing the stink bug egg masses, *Telenomus podisi* and *Ooencyrtus* sp. *Telenomus podisi* was detected on all three different stink bug egg masses,

where *Ooencyrtus sp.* was only detected on *Chinavia halaris* (Say) green stink bug (GSB) egg masses in year one of the survey. Over 80 percent of BMSB egg masses put into the field in the ornamental plantings were predated upon, while under 40 percent of egg masses in the natural habitat were predated upon, and 0 percent of egg masses in corn were predated upon. While the greatest percent of egg masses parasitized was found in the GSB in the natural landscape where over 40 percent of the egg masses were parasitized in year one of the survey. The BMSB only had egg masses parasitized in the ornamental planting landscape during the survey. No parasitism was detected in the corn field.

Chapter 1

Introduction

Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera; Pentatomidae) is an insect native to Asia. It was first discovered in the United States in the mid-1990s near Allentown, Pennsylvania. As of October 2015, the BMSB had been detected in 42 states and two Canadian provinces (APHIS 2015). Populations near the original detection have exploded in recent years causing many problems for a variety of different crops (Leskey et al. 2012). The BMSB is considered an agricultural pest in 15 of the 42 states in which it has been found (APHIS 2015). It was first found in Connecticut in 2008 in West Haven, and has subsequently been found in most towns in the state, encompassing all counties (APHIS 2015). So far the state damage reports are minimal with few instances of significant localized damage to crops. Most reports are due to residents finding the BMSB overwintering in their homes or searching for a place to overwinter. As of summer 2014, the BMSB was labeled as an agricultural pest in the state of Connecticut. Traps have started to collect above threshold levels in the state, in fact crops such as peaches and apples have needed pesticide spraying in August of 2014 and 2015. The BMSB is known to feed on over 70 crops and fruit trees found in Connecticut, not to mention ornamental and landscape plants (Leskey et al. 2012). The BMSB has been observed in other states, and is inflicting large amounts of damage to orchards, corn, and soybeans, but it will feed on many other plants as well (APHIS 2015).

Identification

Typical of other stink bugs, the BMSB has a shield-shaped body and can emit a pungent odor when disturbed. With a mottled brown, 12 to 17 mm long body, it has characteristic alternating dark and light bands across the last two antennal segments that appear as a single white band in both nymphs and adults. This is the most distinguishing characteristic in the field, although it can easily be confused with other Heteropterans, including native brown stink bugs from the genera *Brochymena*, *Euschistus* and *Parabrochymena* (Hobeke and Carter 2003). BMSB can be told apart from other stink bugs in the USA by their larger size, light colored banding on antennae, and legs, and alternating light and dark bands around the abdomen (Leskey et al. 2012). The BMSB can also be distinguished from other stink bugs, and similar species, by its rounded pronotal lateral tips/margins. Females tend to be larger and broader than the smaller males. Both female and male BMSBs have a dark line that goes across the widest part of their upper thorax. These lines typically have light-colored spots along them. In males, these spots are raised to form bumps. In females, they are smooth (Leskey 2013).

Biology

The BMSB has five nymphal stages before reaching the mature adult stage. The BMSB first instar is small (2-3mm), and tends to be reddish/ orange in color. During this stage the nymphs tend to stay around the egg mass they hatched from. First instar nymphs must obtain a gut symbiont, *Pantoea agglomerans* left by the mother on the eggs in order for them to be able to digest food and survive (Taylor et al. 2014). Without this gut symbiont nymphs will not be able to digest food and will die. After each successive molt, the next instar takes on a darker color and starts looking more and more like the adult BMSB (Gyeltshen 2013). First instars nymphs

emerge four to five days after eggs are laid, each instar following tends to last a week, depending on temperature (Gyeltshen 2013).

In Pennsylvania and northern areas, the BMSB is only known to have one generation per year. As the stink bug spreads to southern areas towards warmer climates, it is likely to produce more generations. In its native southern China, the BMSB has up to five generations per year (Leskey et al. 2012). The adults mate in the spring approximately two weeks after emerging from diapause. After a short period, the females begin laying egg masses. Egg masses are laid at approximately weekly intervals, and each female lays as many as 400 eggs in her lifetime (an average of 10-16 egg masses). In Pennsylvania, females were observed laying eggs from June to September (Gyeltshen 2013). Females must eat before being able to lay their light green eggs (Leskey et al. 2012). In late August, triggered by the decreasing day length and lower temperatures, adults begin to congregate in large numbers on hosts plants prior to moving to overwintering sites. These sites include man-made structures, dead and standing trees, and rocky outcrops (Leskey et al. 2012).

In Connecticut, the BMSB has one generation per year and overwinters as adults (O'Donnell and Schaefer 2013). BMSBs in Connecticut become active in the spring once temperatures rise and leaves start opening up, normally in May. The BMSBs feeds and starts laying eggs in June in Connecticut. BMSBs complete development on trees of heaven and peach trees, but also have over 100 other host plants on which they can lay eggs and develop (Rice et al. 2014). The most suitable food plants, and most often plants that have eggs on them, have some sort of fruiting body that is a suitable food source for young nymph stink bugs (Rice et al. 2014). Eggs are normally laid through September, then the BMSB starts searching for overwintering places (Jacobs 2014).

Problems with BMSBs

BMSBs have been observed on over 170 different species of plants in the United States, ranging from vegetable crops, to wild plants, to fruit orchards. It has been estimated that the BMSB feeding can result in losses of 21 billion dollars for the agricultural industry in the United States (Woodsen 2013). Similar to other stink bugs, the nymphs and adults have a piercing-sucking type of mouthparts. In order to obtain the nutrients of the liquid part of the fruit, stink bugs use these mouthparts in a straw-like fashion by piercing the fruit. Stink bugs inject digestive juices into the fruit or plant, thus making more juices available to be taken in by the stink bug. Small necrotic spots on fruit and leaf surfaces often result from feeding damage, and it may be compounded by secondary infections and scarring as the fruit matures (Gyeltshen 2013). The BMSB also outcompetes native stinkbugs, and, once established, they often become the predominant stink bug species in the area (Leskey et al. 2012). BMSBs are also a problem for the nuisance they cause by getting into people's homes to overwinter. One homeowner in Maryland collected over 26,000 BMSB from his house from January to June of 2011 (Leskey et al. 2012).

Although BMSB damage risk to vegetable crops has not been fully determined, corn appears to be the crop at greatest risk and the favorite host plant for the BMSB (Leskey et al. 2012). Some infested corn fields in the mid-Atlantic have been observed with damage on 100% of corn ears (Leskey et al. 2012). Other crops that have experienced over 20% damage caused by BMSB include tomatoes, peppers, eggplant, and okra (Leskey et al. 2012). Other crop groups including cucurbits and crucifers seem to not be favorite hosts of the BMSB (Leskey et al. 2012).

Other Stink Bugs Found in Connecticut

There are 45 species of stink bugs found in Connecticut and 13 of these species are predators. Most of the members of this group are native to the state. The Green Stink Bug is one of the most common and widely found stink bugs in the state, thus it has been selected for this study as well. The Brown Stink Bug is also a very common stink bug found in Connecticut, and is easy to catch with pheromone traps and beat sheet sampling.

The Green Stink Bug

The Green Stink Bug (GSB) *Chinavia hilaris* (Say) is a pest of many seed, vegetable, and fruit crops. It is commonly found throughout North America. GSB is the most commonly found stink bug in the United States (Gyeltshen et al. 2013).

Identification

The GSB can be identified by its bright green color and large size (13-19 mm), narrow body, and straight or almost straight anterolateral pronotal margins. Similar species can be distinguished by the following: the *Chinavia pennsylvanica* (Gmelin) has a broader body. The southern green stink bug, *Nezara viridula*, (Linnaeus) has a short obtuse spine on the second abdominal sternite and a short ostiolar canal (McPherson 2013). The green stink bug goes through 5 instars, starting off black, red and white, with each instar getting larger and gaining more features of the adult green stink bug. The final green color does not show until the fourth instar.

Biology

The GSB overwinters as an adult, preferring to do so in leaf litter and deciduous woodlands (Underhill 1934). It emerges from diapause when temperatures rise above 18°C (McPherson 1982). Adults are most active when temperatures exceed 24°C (Kamminga et al. 2012). The green stink bug has a single generation in northern areas such as Canada (Javahery 1990), but in more favorable southern conditions there may be two generations (Sailer 1953, Kamminga et al. 2009). Females that have overwintered in eastern North America begin laying eggs in the middle of June and end the first week of September, with peak egg laying occurring the third week of July (Underhill 1934, Javahery 1990). The largest populations of first generation egg masses are found near the woods where adults have overwintered. This generation continues to feed on weedy hosts and migrates into nearby crops once they become attractive as a food source (i.e., budding or fruiting structures). Miner (1966) reported that the second generation remains in the crop system throughout its life stages. Females can lay a new egg cluster every 8–10 days. These eggs are deposited vertically in clusters of 1–72 (Underhill 1934) with an average of 32 eggs per egg mass (Miner 1966). However, 130 eggs per cluster have been observed under laboratory conditions (Miner 1966, Javahery 1990). Time between egg mass depositions typically decreases after the first mass is laid (Underhill 1934, Nielsen et al. 2008). Eggs are usually deposited to the underside of leaves. The eggs are barrel-shaped and change from light green to yellow or light pink before hatching in about 1 week (Miner 1966). The duration of the egg stage depends on temperature. Shorter durations occur in warmer temperatures, and longer durations in cooler temperatures (Underhill 1934, Capinera 2001). Upon hatching, they undergo five instars before becoming adults (Underhill 1934, Miner 1966). The first instars do not feed and remain clustered together around the egg mass. Second instars

are more gregarious and begin to feed. Third instars behave in a similar manner to second instars, but are slightly larger in size. Feeding by the fourth and fifth instars can result in as much economic damage to the plants as from adults (McPherson 2012).

The Brown Stink bug

The brown stink bug (BSB) *Euschistus servus* (Say) is a common pest of many seed, grain, fruit, and nut crops, and considered a severe pest in some areas. The BSB is commonly found throughout North America.

Identification

The BSBs, like other stink bugs, are shield shaped as adults and are brown to gray with dark speckling on their backs. The fourth and fifth antennal segments tend to be darker in color. The ventral surface of the BSBs tend to be gray to red or green, depending on what the insects are feeding on. Adult insects range from 10 to 15 mm in length with the females normally being larger (Aldrich et al. 1991).

Biology

The adult BSB overwinters in protected areas such as leaf litter, tree cavities, houses, rock outcrops, and more. BSBs become active in the first warm days of spring normally once temperatures reach 21°C. Since the BSB is active early in the spring, the first generation normally develops on wild plants, while second generation insects develop on more cultivated plants. Females can normally lay eggs within a week of feeding, and over their 100-day lifespan, can lay as many as 18 egg masses with as many as 60 eggs per mass (Virginia Tech 2008). Eggs are normally affixed to the underside of leaves and are translucent green when laid. Over time

the eggs become more yellow and then red before hatching approximately 5 days after being laid. The time spent in the 5 instars prior to becoming adults depends on the temperature, humidity, and availability of food. Much like the GSB and BMSB the nymphs of the BSB start to look more and more like the adult BSB as they progress through each nymphal stage.

BMSB Pest Management Options

Integrated Pest Management (IPM)

IPM is the use of many methods to create the most environmentally safe, and economically feasible approach to limiting pest damage (EPA 2013). The following IPM methods have been considered for BMSB management.

Trap crops

Trap crops are used to either prevent pests from reaching the main crop, or to concentrate pest individuals in a certain area of a field in order to destroy them mechanically or chemically (Knight and Gurr 2007). The principle behind trap crops is that virtually all insects show a preference to a certain plant species, cultivar, or stage of plant (Newsom and Herzog 1977). The exact crop and timing of a trap crop for the BMSB is still under investigation, but initial research shows that BMSB tend to prefer podding soybeans when given choices amongst field crops (Leskey et al. 2012). Planting strips of soybeans early in the season, so they have pods on them when BMSB are emerging (June) is key. Once the BMSBs start becoming attracted to the pods, one could either start spraying or destroying the trap crops mechanically. The problem with destroying the trap crops mechanically is that once they are destroyed there will not be a trap crop for later emerging BMSBs.

Biological Control

Biological control is the use of either introduced or naturally occurring organisms to control an unwanted species. Asian natural enemies are thought to be an important part of keeping the BMSB populations in check in the BMSBs native area (Leskey et al. 2012). In Asia, there are several parasitoid wasps in the genus *Trissolcus* that parasitize the eggs. Work is currently being done in the U.S. to see if any of these wasps would be suitable to bring over to the USA. Recently a possible candidate that was being evaluated in USDA labs as a possible biological control was found in multiple US locations in different parasitoid surveys. *Trissolcus japonicus*, a parasitoid native to Asian areas where the BMSB is also native, has been caught in Maryland. Although the parasitoid has been under quarantine in the United States for study since 2007, it is thought the parasitoid came across on plant material in a parasitized egg mass.

Native natural enemies have also been considered. Through a few preliminary studies in Mid-Atlantic States (Pennsylvania and Maryland) it has been determined that there is a baseline of about 5% of BMSB adults and eggs being attacked by native parasitoids (Leskey et al. 2012). More recent studies showed a wide range of base rates of parasitism on BMSB eggs. Many factors affect parasitism rates. In certain agroecosystems that include corn field plots there has been much greater egg parasitism, with numbers as high as 55% (Leskey et al. 2012). These data suggest that local ecosystems and dynamics play a large role in the available parasitoids that can target the BMSB. There are several native species of tachinid flies, chalcidoid wasps, and various invertebrate predators that have been observed attacking BMSBs (Leskey et al. 2012).

Egg parasitoid wasps occur in several families collectively referred to as the parasitic Hymenoptera. There are thousands of parasitic wasps that cover all the major ecosystems of the Earth, minus the Polar Regions (Austin et al. 2005). In general, parasitic wasps have modified

ovipositors that are stingers, they use these needle like parts to insert an egg (or multiple) into the host eggs (Austin et al. 2005). The larval wasp then hatches out of its egg and eats the contents of the egg it was laid in, then pupates within the egg (Austin et al. 2005). Some parasitic wasps have many host species while others are more limited in the host eggs they will parasitize.

There are several different parasitoid wasp species that are known to attack/ parasitize BMSB eggs that may be found in Connecticut. First is *Telenomus podisi* (Ashmead). This wasp ranges from Brazil to Canada (Krombein et al. 1979). It is known to feed on many species in the Hemiptera order. This wasp has been observed in crop fields in spring. The next parasitoid wasp of interest is *Trissolcus basalis* (Wollaston). This wasp is known for being the primary controller of the southern green stink bug, but has been seen parasitizing BMSB eggs. *T. basalis* adults mate immediately after emerging from host eggs. The female typically inserts one egg into a host egg. Heaviest parasitoid egg production occurs during the first few days after emergence, and then tapers off. The average number of eggs produced per female was 230-300 in one laboratory study (Cornell 2013). Adults emerge from the host eggs in 9 to 12 days (Cornell 2013). Another possible BMSB parasitoid is *Trissolcus edessae* (Fouts). There is little known about this parasitoid besides the fact that it has been observed on several hemipteran species and has been found as far north as Massachusetts (Krombein et al. 1979). The last parasitoid that may be detected in Connecticut is *Trissolcus euschisti* (Ashmead), this wasp is found as far north as Canada and down into South America. It is an egg parasitoid of stink bugs and related groups. The wasp larva that hatches then feeds on the contents of the egg, pupates inside of it, and then chews its way out as an adult. A female wasp often will stay with the host egg mass after she has parasitized every stink bug egg. She will chase away any other parasitic wasps that might come

to investigate the mass. They are typically docile when they are guarding the egg mass, and it takes quite a bit of disturbance to get them to fly away (Orr 1988).



Figure 1: A parasitoid (see red box) next to a green stink bug egg mass.

Other natural enemies include entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium brunneum*) which have been reported affecting BMSB in its native range and have shown efficacy in lab studies (Sasaki et al. 2012). As is the case with other natural enemies of the BMSB, it is possible to improve the habitat for the fungi, in order to use it as a control. Thus, the goal is to improve the chance for transfer of the fungi to the BMSB. Higher humidity levels allow for greater fungal growth. Populations of fungi are also found more prevalent in low or no till soils, since the structure has been less disturbed (Knight and Gurr 2007). Lower tilled soils also tend to have greater organic matter in the soil and thus hold more moisture for fungi to thrive (Knight and Gurr 2007). Decreasing row spacing in crops could also increase fungal activity in the soil, with decreased row spacing there is a greater canopy over the soil allowing for less evaporation from the soil (Knight and Gurr 2007).

Insecticides

Insecticides are commonly used for the management of stink bugs, specifically broad spectrum pyrethroids (Koppel et al. 2009). The problem with these insecticides is that they do not only kill stinkbugs, but they also kill the previously mentioned beneficial insects (Koppel et

al. 2009). The use of broad spectrum insecticides causes a chain reaction in an ecosystem, sometimes resulting in a flare up of secondary pest insects. In 2011 throughout the mid-Atlantic region, the amount of pesticides used was increased four times the amount used in previous years in certain crops (Leskey et al. 2012). This practice disturbed IPM practices and caused outbreaks of normally controlled pests including European red mites, woolly apple aphids, and several types of scale insects (Leskey et al. 2012).

The conservation of beneficial insects could be done by choosing less broad spectrum insecticides, insecticides that are less harmful to the beneficial insects that naturally reduce BMSB populations (Koppel et al. 2009). The use of "softer" insecticides can be effective at controlling nymph stages of the BMSB. These softer insecticides are also less harmful to beneficial insects (Koppel et al. 2009). An example of a softer insecticide would be insecticidal soaps. These soaps kill on contact via suffocation and do not cause long term residual effects on other insects. In order for soaps to be successful more research is needed to figure out the optimal timing for spraying to maximize their effectiveness. Another option would be to limit the area treated. For example, in surveys from 2011, it was found that highest BMSB populations were found along field edges. Thus treating just 12 meters into the field prevented further invasion and resulted in 85%-95% reduction in insecticide used when compared to whole field treatment (Leskey et al. 2012).

The following is a table of active ingredients that have produced significant mortality rates in BMSB under laboratory conditions.

Table 1: Insecticides recommended for BMSB management

| Active Ingredient | Residential Use | Ornamental Trees/Shrubs | Vegetables |
|--------------------------|------------------------|--------------------------------|-------------------|
| Acetamiprid | No | Yes | Yes |
| β-cyfluthrin | Yes | Yes | No |
| Bifenthrin | Yes | Yes | No/Yes |
| Cyfluthrin | Yes | Yes | No/Yes |
| Deltamethrin | Yes | Yes | No |
| Dinotefuran | No | Yes | No/Yes |
| Fenpropathrin | No | No | No |
| A-cyhalothrin | Yes | Yes | No/Yes |

Adapted from (Rutgers 2014).

Sterile Insect Technique

This concept involves releasing a large enough number of sterile male insects to prevent reproduction and start a downward trend in the population (Knight and Gurr 2007). Insects are captured and exposed to ionizing radiation thus making them infertile. Populations of these infertile insects are then released creating a less fit population (Leskey et al. 2012). This method used for other stink bugs has been found to not be economically feasible since it is expensive to raise large amount of stink bugs for simultaneous release (Knight and Gurr 2007).

Current research needs and project objectives

My project comprised two separate, yet equally important experiments. First a parasitoid survey was completed, in which a baseline of the native parasitoids that exist in Connecticut was determined. Over two different summers' two native stink bugs and the BMSB were reared in colonies and their egg masses were put into the field in three different habitat types. Ideally this research would help USDA researchers determine if a traditional biocontrol agent was needed or if there is a possibility of enhancing a native parasitoid species to help control the BMSB population. There is an urgent need for more information on how to control this pest, because little is known about what the natural enemies of the BMSB are, and specifically what

parasitoids attack BMSB egg masses here in Connecticut and in other New England areas. Since the egg stage is the most vulnerable stage in the stink bug's life cycle, figuring out what exactly attacks BMSB eggs and at what rate would provide a baseline as to what percentage of insects will need to be controlled with other methods. The identification of specific predators and parasitoids could also lead to techniques to try to increase predation rates, or manipulate parasitoid presence.

The second project had a goal of improving the rearing and health of the stink bug colonies maintained in the lab. This experiment was run twice, and individual stink bugs were followed from birth to death while being put on one of several different diets. This research aimed to create a better understanding lab rearing diets using food options available to other scientists who use lab reared colonies of BMSBs for similar or different studies. Being able to use lab reared insects versus wild caught stink bugs cuts down on experimental variables.

Colony maintenance is extremely important because there are many teams performing research on the BMSB in the different states where the BMSB has been found. For example, the USDA is conducting research on exotic parasitoids for the biological control of the BMSB. Information is also needed on native predators and parasitoids that could attack this pest. Therefore, the ability to rear healthy colonies is a necessity to further the research that is so highly needed.

Chapter 2

Stink Bug Egg Parasitoid Survey

Introduction

There is little information known about the parasitoids found in Connecticut that attack the native stink bugs and the invasive BMSB. The most viable option for controlling stink bugs is during their most vulnerable stage, the egg stage, where stink bugs are not mobile or able to protect themselves. In this project, a survey of native parasitoids was done using sentinel egg masses over two growing seasons from three different stink bug species to detect the presence of different egg parasitoids of stink bugs.



Figure 2: *Telenomus podisi* hatching from a brown marmorated stink bug egg.

Objectives

The first objective was to determine what parasitoids native to Connecticut attack the BMSB's egg masses. This question was answered by putting out sentinel egg masses of the BMSB. The second objective was to survey egg parasitoids of GSB and BSB. Obtaining information on not only the invasive BMSB, but also the native stink bugs allows inferences to

be made, such as determining if certain parasitoids have a preference for a particular stink bug species. Egg masses of all three species were put out at the same locations to determine if predators or parasitoids preferred the native BSB or the non-native BMSB. And as a third objective, the influence of habitat was investigated by placing egg masses in a natural habitat, ornamental habitat, and field corn habitat. Each different habitat had different host plants that the egg masses were placed on. These plants were chosen because of the representative nature of the habitat as a whole. The last objective was to detect when in the growing season parasitoids were most active, or at their peak. In the second season, egg masses were put out from June through August to try to detect the peak of activity, and when the greatest chance for egg parasitism occurs in Connecticut.

Methods

Considering that populations of BMSB are low in the state, all egg masses used in this project were reared from colonies and deployed as sentinel eggs. GSBs were also raised in colonies for ease of access. Colonies were started from trapped stink bugs. Once laboratory populations were determined to be large enough, egg masses were collected and put into the field. Colonies were reared in cages with different types of substrate in them for egg laying, including a living plant in all colonies at all times. Bush bean, *Phaseolus vulgaris* "Tendergreen" (New England Seed Company) plants were grown in the greenhouse and new plants were started on a continuous schedule so there was always a podding plant for each cage.

Colonies were set up in screened cages with mesh sleeve doors. Enclosures were kept in incubators at 25°C with relative humidity maintained at a high level by putting a large tray of water at the bottom of the incubator. Once incubators were filled additional cages were maintained in a greenhouse mist room. The mist room was maintained between 21°C and 28°C.

The misters were set off depending on incoming sunlight, meaning during hot summer days with bright sun the misters could go on as often as every five minutes or as little as once every hour. This sporadic misting kept humidity in the greenhouse relatively high most of the time. Cages were limited to 15 insects per cage while trying to keep sex ratio as close to 1:1 as possible. GSB cages were limited to 10 insects per cage. Die off tended to occur if more insects were added to the cages. Each enclosure contained a mature podding bean plant and a mix of whatever was available for fresh organic produce. Apples, peaches, peppers, beans, carrots, and autumn olive were used as food sources over the two years. Each enclosure also contained several different egg laying surfaces in addition to the bean plant including sheets of wax paper, crumpled computer paper, and mesh were hung in the cages. Initial populations of stink bugs were caught by using beating sheets, black light traps, and lure traps using USDA #10 lures, AgBio stink bug lures, and Sterling Rescue stink bug lures. Black light traps were best at catching GSBs, lure traps yielded the most BSB and BMSBs in the spring, early summer, and late summer when BMSBs were moving from the woods to the fields and then from the fields back to overwintering locations. Beat sheet sampling yielded a good variety of different insects, in certain locations beat sheets worked very well for catching stink bugs. Beat sheet samples were also vital in telling when stink bugs had emerged from overwintering. Cages were checked for eggs daily. Initial eggs were collected and placed in petri dishes in an incubator. The petri dish top lid was misted daily to maintain high humidity for the eggs. Once eggs hatched and the stink bugs left the egg masses, insects were thinned down to smaller numbers in containers with other food sources. When insects reached adulthood they were added to cages. Once the rearing colonies reached adequate numbers eggs started to be removed for placement into the field. Some egg masses continued to be retained in the incubator to maintain rearing colonies. Eggs

collected for the field were put into petri dishes and then placed in a zip lock bag and placed in a freezer at -17°C. Eggs were labeled with information including number of eggs, date of collection, and species. When there were enough eggs to start placing in the field, eggs were adhered to ¼ of an index card with Duct Tape brand double sided sticky tape (2014) and Elmer's scrap book glue squares (2015). All handling of egg masses was done using gloves; gloves were changed when handling different species. If egg masses were laid on leaves or some other surface, the surface was cut as close to the egg mass as possible without harming the egg mass. Photos of the egg masses were taken in case further information about the egg mass was needed at a later time. Egg masses were sprinkled with Quikrete® play sand after the picture was taken in order to cover any sticky surfaces left from the double sided tape or glue square. This prevented any predators or parasitoids from getting stuck on the sticky surface.

In 2014, egg masses were put out from July 17th to August 26th, in 2015 egg masses were put out weekly from June through August in each of the three habitat types except for corn, where the first egg masses were not set out until the first week of July when the corn was big enough. In 2015, egg masses were put out weekly with a paired set of egg masses, one egg mass from BMSB and one from BSB in close branch vicinity. Egg masses were placed within plants approximately three to four feet off the ground. In the ornamental planting the egg masses were placed on hydrangea, flowering dogwood and swamp dogwood. In the natural habitat the eggs were placed in multiflora rose and alder trees. In the corn habitat the egg masses were placed in corn. The egg masses were attached to the underside of the leaves of these plants using insect pins weaving the pins between the index card and plant leaves. In corn, toothpicks (colored black to look similar to the pins) were used to weave the index cards and corn leaves together so

if a pin was lost it would not set off the metal detector on the combine. Egg masses were left in the field for 48 hours.

The following sites were used for deploying sentinel egg masses:

All sites used were in Storrs Mansfield, Connecticut and within two miles of each other.

University of Connecticut Plant Science and Education Research Facility

1015A Agronomy Road Storrs, CT 06068

- Field corn: In year one the field corn used was located at the UConn research farm and was planted by Dr. Morris. The corn variety used was DeKalb DKC45-82, this is a Round-Up® ready variety.
- Nursery crop plantings: Area of different ornamental plants. In year one the planting was weedy, but year two the planting was weeded and covered in a natural pine mulch. The planting contained a wide variety of ornamental plants including several different pine species, dogwood species, and flowering trees. Eggs were attached to flowering dogwood and hydrangea.
- Natural areas: Forest edges. The forest edge used had a good mix of plants including some invasive plants including autumn olive and multiflora rose. The forest edge also contained many native species including alder, red oak, gray birch, red maple, sugar maple, and muscle wood, along with many open field grasses.

University of Connecticut Corn fields

- In year two, south of the UConn research farm on Route 195, field corn, which had the same DeKalb DKC45-82, Round-Up® ready corn used in 2014. Located at 41.780776 - 72.222266.

After 48 hours the masses were approached cautiously as to not disturb any predators or parasitoids that might still have been on the egg masses. If any predators or parasitoids were seen, an attempt was made to catch them with an aspirator or other means. After egg masses were removed from the field another photograph was taken of each egg mass. Missing and preyed upon eggs were recorded. If any eggs were missing or predated upon the type of predator damage was also recorded as, either pierce-sucking or chewing damage. Egg masses were then returned to their petri dish and placed in the incubators. They were checked regularly and the tops of the petri dishes were sprayed with water every couple of days to maintain humidity. If parasitoids hatched out of egg masses they were allowed to move around the petri dish. The parasitoids were collected after they died in the petri dishes and put into vials containing 90% ethanol. Parasitoids were then sent to Dr. Christine Dieckhoff, USDA, Beneficial Insect Laboratory for identification. After about a month, remaining eggs that had not been preyed upon or hatched a parasitoid were dissected under a microscope. The best method for dissecting the eggs was to cut a slit in the top of the egg with a scalpel and then squeeze the bottom on the eggs with a pair of forceps. Alcohol based hand sanitizer was used on the eggs to prevent them from leaving the petri dish and getting lost while dissecting the egg masses.

Statistical analysis was done using SAS version 9.4. Chi-square tests were done to determine if there were significant associations between habitats and predation / parasitism.

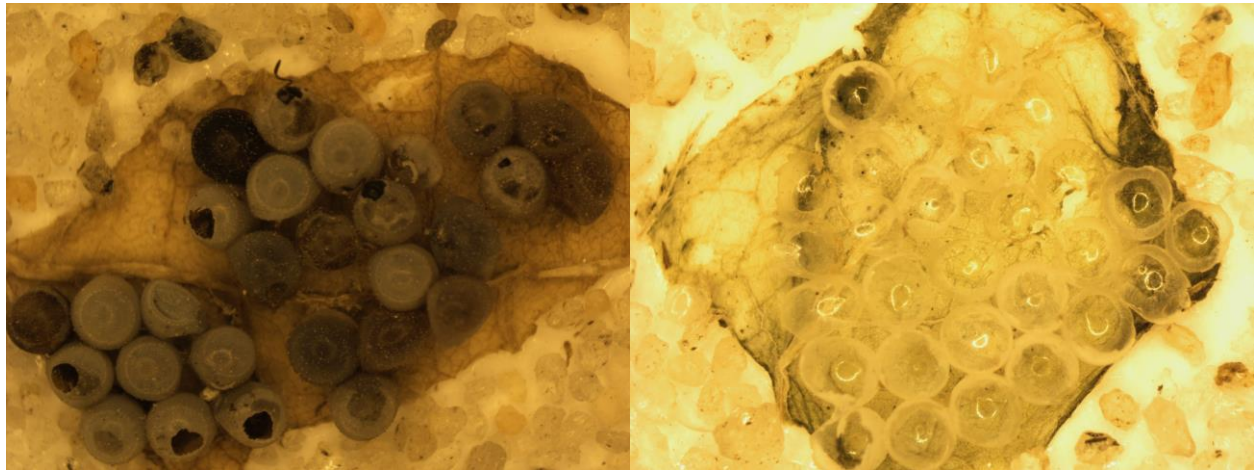


Figure 3: Brown marmorated stink bug egg masses and types of natural enemy damage. The difference between eggs on the left that were parasitized (some parasitoids still working their way out) versus egg mass that was completely eaten by predators (right).



Figure 4: A brown stink bug and brown marmorated stink bug egg mass attached to a leaf blade of corn.

Results

Over the course of two summer field seasons (2014 and 2015) two different parasitoids were collected on three different types of stink bugs (Table 2). In the two seasons over 2,200 eggs were put into the field comprising some 117 individual egg masses. These eggs yielded 62 individual parasitoids and over 200 predated eggs. Eggs were placed in three different habitats ornamental, natural, and corn. In the corn there was no detected predation or parasitism in either year (Figure 5). Overall, in each habitat with each of the three species predation occurred more often than parasitism except for GSB in the natural habitat where parasitism occurred more often than predation. GSB also had the most overall parasitoids collected from its egg masses.

In general, significantly more eggs were subject to predation upon in the ornamental habitat than the other two habitats across all three species (Figure 5 and Table 4). Parasitism rates were highest in the natural habitat for the two native stink bugs tested (Table 4). The BMSB had no parasitism detected in the natural habitat.

Many egg masses had a few eggs predated upon or parasitized, but very few egg masses were completely eaten or parasitized (Figure 6). Differences between rates of chewing predators versus piercing sucking predators were seen on eggs of all three stink bug species eggs, there were consistently more eggs chewed than pierced and sucked (Table 3).

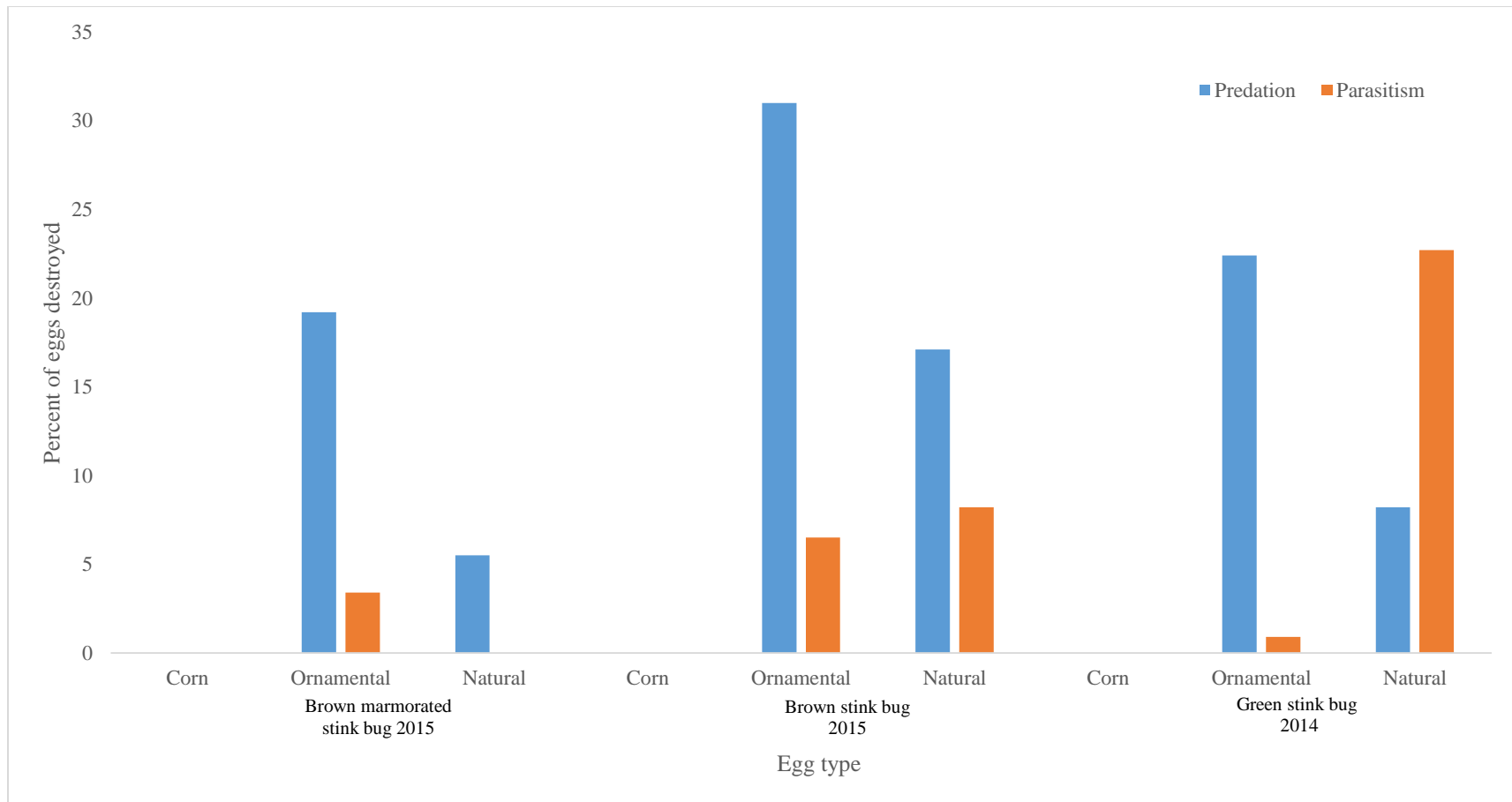


Figure 5: Percent of individual eggs destroyed due to predation or parasitism in three habitat types for three different stink bug egg species.

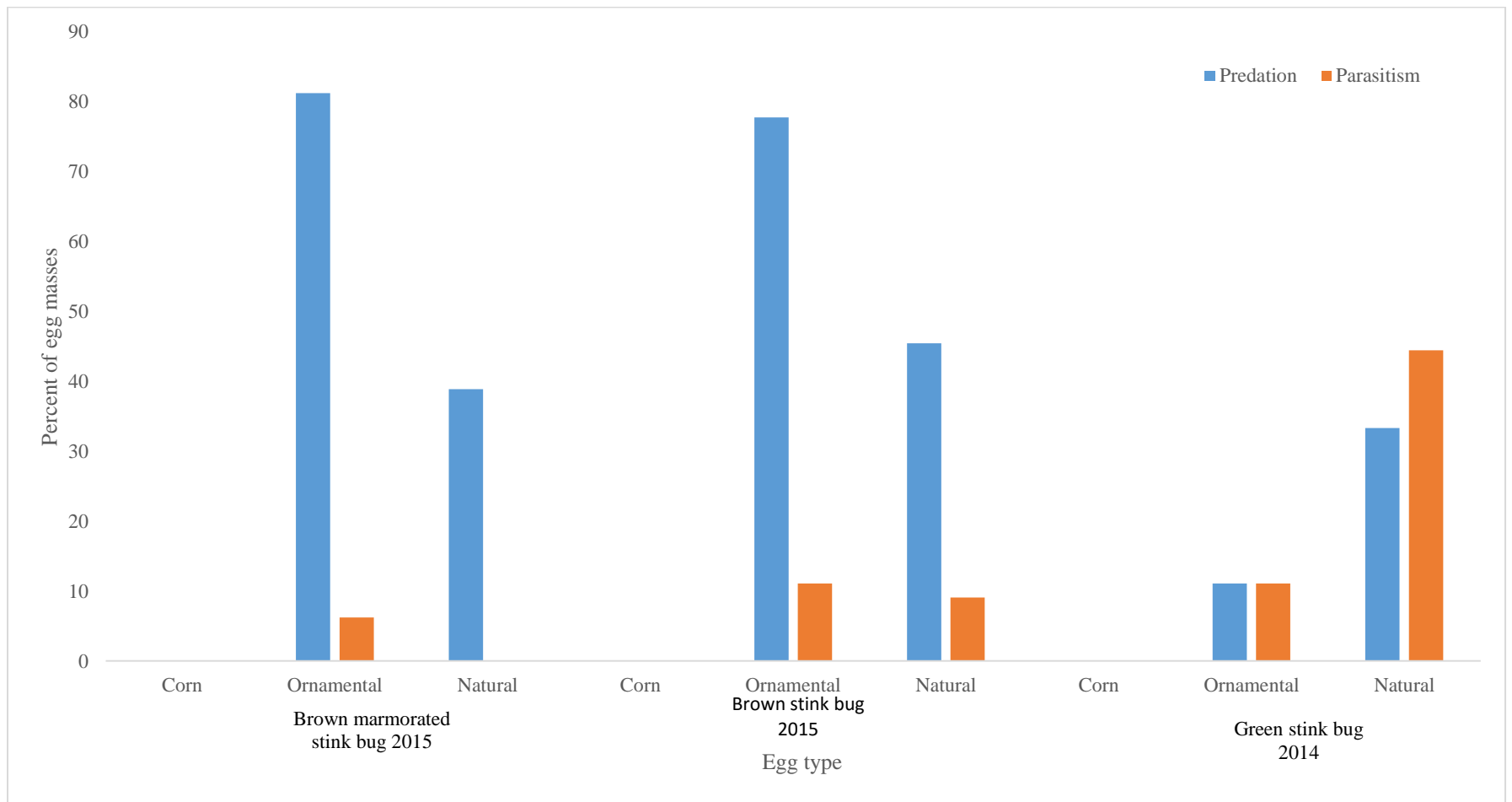


Figure 6: Percent of stink bug egg masses attacked by parasitoids and predators in 3 habitat types.

Table 2: Parasitoids species detected by sentinel egg host

| Parasitoid | Host | Number of Eggs Parasitized | Number of Eggs Hatched | Percent Hatched | Average Time to hatch (days) |
|-------------------------|----------------------------|-----------------------------------|-------------------------------|------------------------|-------------------------------------|
| <i>Ooencyrtus sp.</i> | Green stink bug | 2 | 0 | 0% | - |
| <i>Telenomus podisi</i> | Green stink bug | 35 | 25 | 71.4% | 21.24 |
| | Brown stink bug | 21 | 17 | 81% | 20.57 |
| | Brown marmorated stink bug | 16 | 11 | 68.8% | 22.67 |

Table 3: Predation damage type detected on stink bug sentinel eggs

| Stink bug species | Piercing sucking predation¹ | Chewing Predation |
|----------------------------|---|--------------------------|
| Green stink bug | 3 | 33 |
| Brown stink bug | 14 | 58 |
| Brown Marmorated stink bug | 37 | 77 |

1: Number of eggs affected

Table 4: Influence of habitat type on predation and parasitism of sentinel stink bug eggs

| Predation | | | | | |
|----------------------------|------------|-------------|----|----------------|-------------|
| Stink Bug | Habitat | % Predation | DF | X ² | Probability |
| Green stink bug | Natural | 8.4 | 1 | 10.22 | 0.0014 |
| | Ornamental | 22.4 | | | |
| Brown stink bug | Natural | 17.1 | 1 | 8.32 | 0.0039 |
| | Ornamental | 31 | | | |
| Brown marmorated stink bug | Natural | 5.5 | 1 | 42.62 | <0.0001 |
| | Ornamental | 19.2 | | | |
| Parasitism | | | | | |
| Green stink bug | Natural | 22.7 | 1 | 23.86 | <0.0001 |
| | Ornamental | 0.9 | | | |
| Brown stink bug | Natural | 8.2 | 1 | 0.23 | 0.6309 |
| | Ornamental | 6.5 | | | |
| Brown marmorated stink bug | Natural | 0 | 1 | 19.84 | <0.0001 |
| | Ornamental | 3.4 | | | |

Discussion:

During the two years of the survey three different stink bug species were used. The BMSB and GSB were used in the summer of 2014, with limited number of BMSB egg masses put out. In the summer of 2015 the two stink bug species used were the BSB and BMSB. Over the two years there were two different stink bug egg parasitoids detected. *Telenomus podisi* (Ashmead) was detected in 2014 on GSB egg masses and was also detected in 2015 on BSB and BMSB egg masses. *T. podisi* was detected on BSB and GSB in the natural and ornamental planting and only in the ornamental planting for BMSBs. *Ooencyrtus sp.* was detected in 2014 on a GSB egg mass. This individual egg that was parasitized had two wasp individuals inside it, which is probably why the egg did not hatch. The egg mass that yielded the *Ooencyrtus* was placed in the field in early August in the natural landscape. *Telenomus podisi* (Ashmead), is

known as a broad spectrum stink bug parasitoid. *Telenomus* is found in the superfamily Platygastroidea and within the family Scelionidae. Within this family there are an estimated 7000 species (Marshall 2009). The most commonly found species in *Ooencyrtus* in the eastern United States is *Ooencyrtus kuvanae*, which was introduced in to the United States in 1909 from Japan in order to control gypsy moths (Marshall 2009). *Ooencyrtus* is known as a broad spectrum parasitoid as apparent by the parasitoid being brought over to control a lepidopteran and in this study it was detected on a hemipteran species. *Ooencyrtus* still plays a vital role in keeping the gypsy moth populations in Eastern United States in check (Marshall 2009).

Another broader study that used sentinel egg masses for parasitoid detection (Ogburn et al. 2016) detected 8 different parasitoids, 6 of which were found on BMSBs. *T. podisi* was by far the most common parasitoids discovered on native stink bugs and accounted for just under half the total number parasitoids detected on BMSBs. The second most common parasitoid identified was *Ooencyrtus* on BMSB. Although *Ooencyrtus* was not detected on BMSB here in Connecticut, this study detected the two most common parasitoids found in a similar study conducted in Kentucky, Michigan, North Carolina, New Jersey, Ohio, Tennessee, and West Virginia.

On average more predation than parasitism occurred in all habitat types with the exception of year one when more parasitism was seen on the GSB in the natural habitat. Overall GSB and BSB egg masses suffered the majority of parasitism in the natural habitat. There was a significantly greater amount of predation seen in the ornamental planting across all three species of stink bugs. It was determined from the field tests that there was a significant association between the natural and ornamental habitat for both predation and parasitism in all three of the stink bug species except comparing the natural and ornamental habitat for the brown stink bug

when dealing with parasitism (Table 4). The distance between the ornamental planting and natural habitat was not very far, but the difference in plant species found in the habitats was drastic. With a greater variety of plants in the ornamental plantings one would expect different predatory insects that could possibly consume the stink bug eggs. The natural areas had more open areas and flowering perennials which could be alternative food sources for parasitoids. BMSB eggs are larger in size compared to the native stink bug eggs, which could play a role in why there were fewer BMSB eggs parasitized. During the two years of putting eggs into the field there was no documented predation or parasitism of any of the stink bug species in the field corn habitat. Although BMSBs are known to be big pests of corn it appears that maybe the monoculture of corn makes it hard for predators and parasitoids to find the egg masses. Similar to this study, Ogburn et al. (2016) saw a much larger amount of eggs damaged by predation compared to parasitism in all habitats. Parasitism rates across other states and habitats ranged from 0 percent to 12.6 percent parasitized eggs in BMSB, GSB, and BSB.

In the limited sample taken by this study, significantly higher parasitoid hatch rates were observed on all three stink bug species compared to other recent studies. Many recent studies including Ogburn et al. 2016; Haye et al. 2015; and Dobson 2015; had low parasitoid hatch rates around or below 50 percent especially when BMSB was the host egg. The difference of the BMSB eggs compared to the native stink bug eggs may be the reason for the lower hatch rates compared to the native stink bugs egg. The BMSB appears to have larger eggs, they may also have different shell thickness, and/or chemical composition. Although the BMSB has the lowest percentage of hatch success of the three-stinkbug species it still has a hatch rate of 68.3%. Other above mentioned surveys across different states yielded native stink bug species hosting parasitoid hatch rates from 90% to 38% hatch rate. Since these relationships have most likely

evolved together it would be assumed that the hatch rates for parasitoids from native stink bugs would be towards the higher end like 71 and 81 percent rates found for *T. podisi* on BSB and GSB eggs in this study. Some of the variance in hatch rates from other studies is probably due to inadequate conditions for hatching. Data from other states reported in Ogburn et al. (2016) had hatch rates for BMSB egg masses below 33% and very few above 50%. This study produced a 68% hatch rate of *T. podisi* from BMSB egg masses. Sample size in many studies as is the case in this study tend to be small, especially with parasitoids hatched from BMSB eggs.

The bulk of the predation seen on all three of the stink bug species was chewing versus the less common piercing sucking predation. Although if one looks at the number of egg masses effected by each type instead of the total number of eggs eaten the difference becomes smaller. In general a chewing predator seemed to be more likely to eat the whole egg mass where a piercing sucking predator normally ate one or a couple of eggs. Most states from Ogburn et al. 2016 had similar results with most of the predation being chewing, except in New Jersey where a majority of the predation was sucking predation. Dobson (2015) also reported low rates of sucking predation compared to chewing predation in Kentucky.

There are still many questions that could be answered by future research. Greater number of replicates could be done to increase chance of having egg masses parasitized by less common parasitoids. Further pairing of egg masses of native stink bugs and the BMSB could be put out into different habitats to see if there is any preference between native and non-native egg masses. The method in which parasitoids find stink bug egg masses could be looked at to see if there is any manipulation that could be done to increase parasitism rates. There is still much to be learned about the new relationships forming between the BMSB and its new habitat and enemies.

Chapter 3

Influence of lab rearing diets on brown marmorated stink bug development & survival

Introduction

Although many researchers rear and maintain colonies of BMSBs, there is little literature as to what diets work best for colony maintenance and growth. Most studies, including Taylor et al, Funayama, and Saski et al. have looked at cohorts of insects on certain natural and lab made diets. In this study, two diet trials were performed following individual insects from hatching to death, over a two year span. Diets consisted of either one or two food items. In both year's carrots, apples, beans, apple bean, apple carrot, and carrot bean were used as food options, while autumn olive fruit was used only in the first year of trials. Currently, lab-reared colonies are maintained in large cages where eggs are removed regularly and hatched separately. Due to the nymph's inherent vulnerability during and after molting, nymphs are not reintroduced into the colonies until they have reached adulthood.

Objectives

BMSB lab diet trials were conducted with the following objectives. First, to determine if there was a regularly available year round food source that could be used to maintain and rear BMSBs. This is important because many local grocery stores do not carry a high variety of organic produce through the whole year. This study evaluated food items that were most consistently available and affordable; for example, organic carrots were regularly available all year round. As a second objective, the experiment sought to determine if there was a nutritional advantage to using multiple food items versus a single item. This study was designed to test the influence on BMSB development when fed on either one of the three main food choices or three

mixed food choices. The trials included apple, bean, and carrot food treatments plus three combination treatments of these foods. The effect of the treatments on insect development speed and overall survival was examined.

Methods

This experiment was conducted from September through December 2014, and October 2015 through January 2016. Cages were regularly checked for BMSB egg masses. Egg masses were collected and placed in petri dishes in an incubator set to 24°C and 14:10 L:D photoperiod. Egg masses ranged from 25-29 eggs with an average of 27.2 eggs per mass. The tops of petri dishes were misted regularly to maintain humidity within petri dishes. Once eggs hatched the nymphs were checked daily, and remained in the petri dishes until they left the egg mass. This normally occurred shortly before 1st instars became 2nd instars or right after becoming 2nd instars, or approximately 5-6 days after hatching. Once the stink bugs left their egg mass they were randomly put in a 2 oz plastic cup in year one or in a 4 oz plastic cup with lid in year two, via the use of a soft-haired paint brush. A notable change in the methods for this experiment was the increase in cup size between year one and two. In year one there was difficulty with moisture control, and overall there was a lack of space and therefore restricted motility for the BMSBs. This is the reason for the increase in cup size. Each lid had four holes punched in it for air circulation and moisture control. The contents of the cups included half of a normal size cotton ball (White Cloud brand) and a random diet choice. In year one, the diets included green bean, carrot, apple, apple and green bean, apple and carrot, green bean and carrot, and autumn olive. In year two the diets included green bean, carrot, apple, apple and green bean, apple and carrot, green bean and carrot, and pepper. The carrots used were Organic Circle baby carrots and the apples used were Rainier Gala apples purchased from Big Y Supermarket. The organic string

beans were purchased from the Willimantic Food Co-op and the source location of the green beans changed regularly depending on the time of year. The organic red bell peppers used were from Trader Joe's or grown at the UConn research farm. The autumn olive fruit were collected from shrubs around UConn Storrs campus and the UConn Depot campus. All food items were refrigerated to increase longevity while not being used. Approximately 5 grams of food were given per individual per food change. Food was changed every three days; if a lot of waste had accumulated in the bottom of the cup, it was wiped out using the cotton ball and a new cotton ball was put in the cup. If there was mold growth in the cup, a new cup would be set up. In 2014 between 23 and 29 individuals were put on each treatment. In 2015 between 29 and 39 individuals were put on each treatment. BMSBs were kept in an incubator set to 24°C and 14:10 L: D. The cups were placed randomly into plastic trays, and these trays were randomly put on shelves within the incubator. BMSBs were checked daily for molting and survival. Recordings were made if molting or death occurred. Once adulthood was reached gender and weight were recorded. Statistical analysis was done using SAS version 9.4. The LIFETEST procedure was used for the log-rank test and Bonferroni multiple comparison adjustment while the PDMIX procedure was used for the analysis of variance (ANOVA), and the Tukey multiple comparison adjustment. The macro program Danda version 2.12 was also used with the PDMIX procedure. The log rank test was done to determine if there were significant differences in the survival curves from each diet treatment, while ANOVAs were done to determine if there was significant difference due to diet treatments, on survival time plus time to adulthood. Data used in ANOVA was examined to determine if they met normality, and homogeneity of variance assumptions.



Figure 7: Setup of treatments for BMSB diet trials in 2015.

Results

Insects fed on apple carrot, apple, bean carrot, and carrot had significantly longer survival than the other three treatments in 2014, while insects fed on green bean, autumn olive, and bean carrot had overall significantly shorter periods between molting in 2014. The experiment setup was greatly improved in 2015. Overall, survival rates and development to adulthood was much more successful in 2015. Treatments that included carrots led to the longest overall BMSB survival, while BMSBs on diets that included beans tended to have the shortest time between molts. Although pepper is a regularly available organic produce item at many local grocery stores, it was not included in any of the later analyses. The pepper had a tendency to spoil very rapidly once cut up and exposed to warm temperatures. Most nymphs placed on the pepper treatment did not survive. Lab-rearing diets have a significant effect on BMSB development and survival. Survival decreases over time as the insects develop toward maturity (Figures 9 and 13). It is worth noting that for the apple treatment, no BMSB made it beyond 4th instar, and for the apple bean treatment no BMSB made it beyond the 5th instar to adulthood (Figures 11 and 12). Carrot treatments had the best overall survival percentage of nymphs progressing to adulthood in both 2014 and 2015 (Figures 9 and 13). The Lifetest procedure using the log rank analysis

indicated significant differences in the survival functions derived from each treatment (Table 6). The Bonferroni adjustment results showed that there were significant differences in the survival functions between some of the treatments in both years (Tables 7 and 8). Overall survival time for insects once placed on a treatment ranged from 3 days to 162 days.

BMSBs placed on an apple carrot diet on average lived the longest, but this is not statistically different from apple, bean carrot, and carrot diets (Figures 8 and 12). However, insect survival on apple carrot is significantly different from those fed on apple bean, bean, and autumn olive diets (Figures 8 and 12). Comparing 2014 to 2015, overall both showed very similar results. In some instances there were more significant differences due to the larger number of individuals used in 2015 (between 0-15 more individuals depending on the treatment).

Individuals who fed on bean, bean carrot, or autumn olive (2014) took the shortest amount of time on average to reach adulthood (Figures 10 and 14). In addition, instar development was impacted by diet treatment. Time between molts was shortest for the bean carrot diet, and autumn olive diet while BMSBs feeding on apple carrot had the longest time to each of the next molts (Figures 11 and 15).

2014:

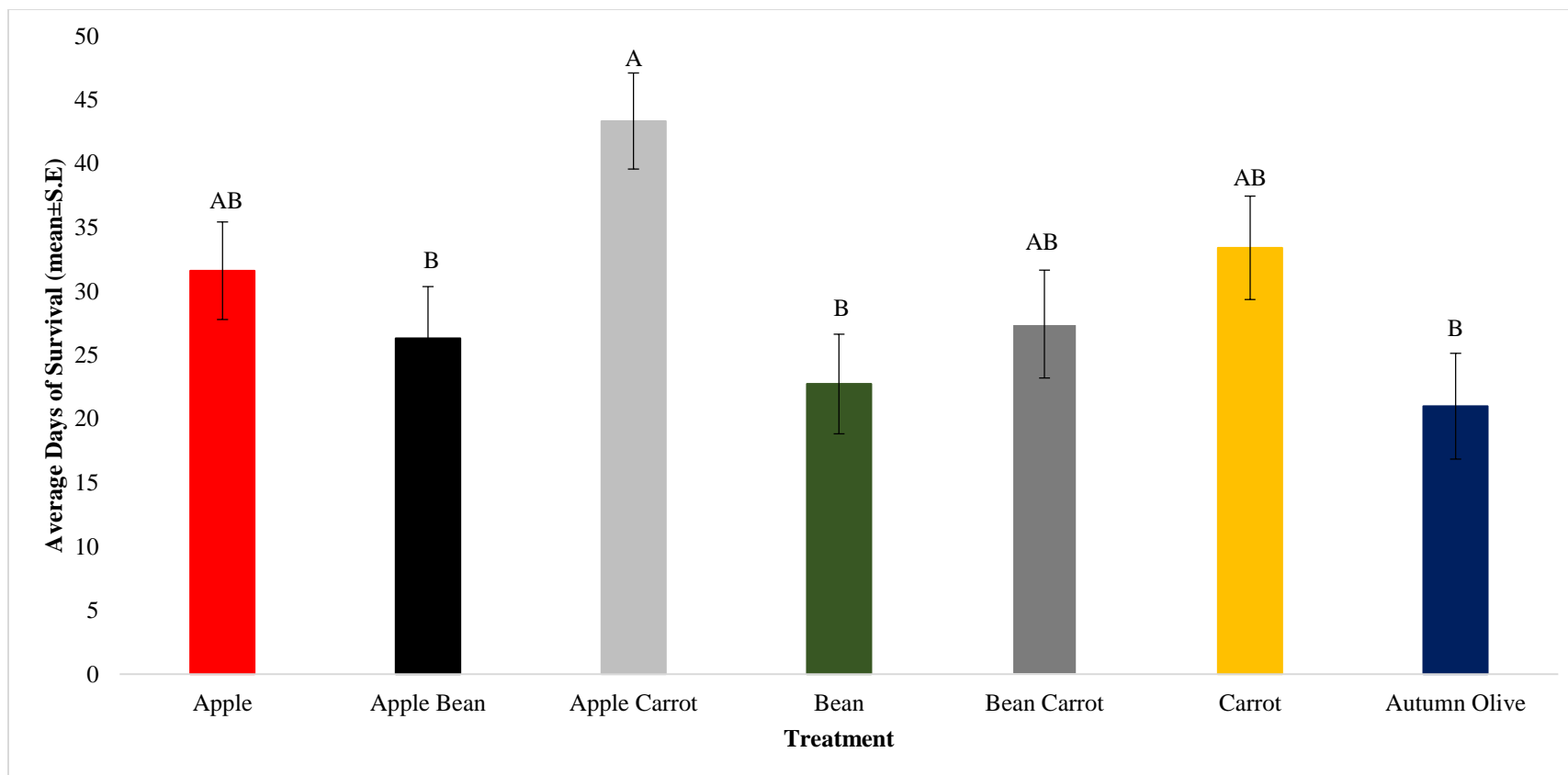


Figure 8: Influence of diet treatment on brown marmorated stink bug survival in 2014 diet trial. Bars with the same letter are not significantly different according to Tukey's test ($\alpha=0.05$).

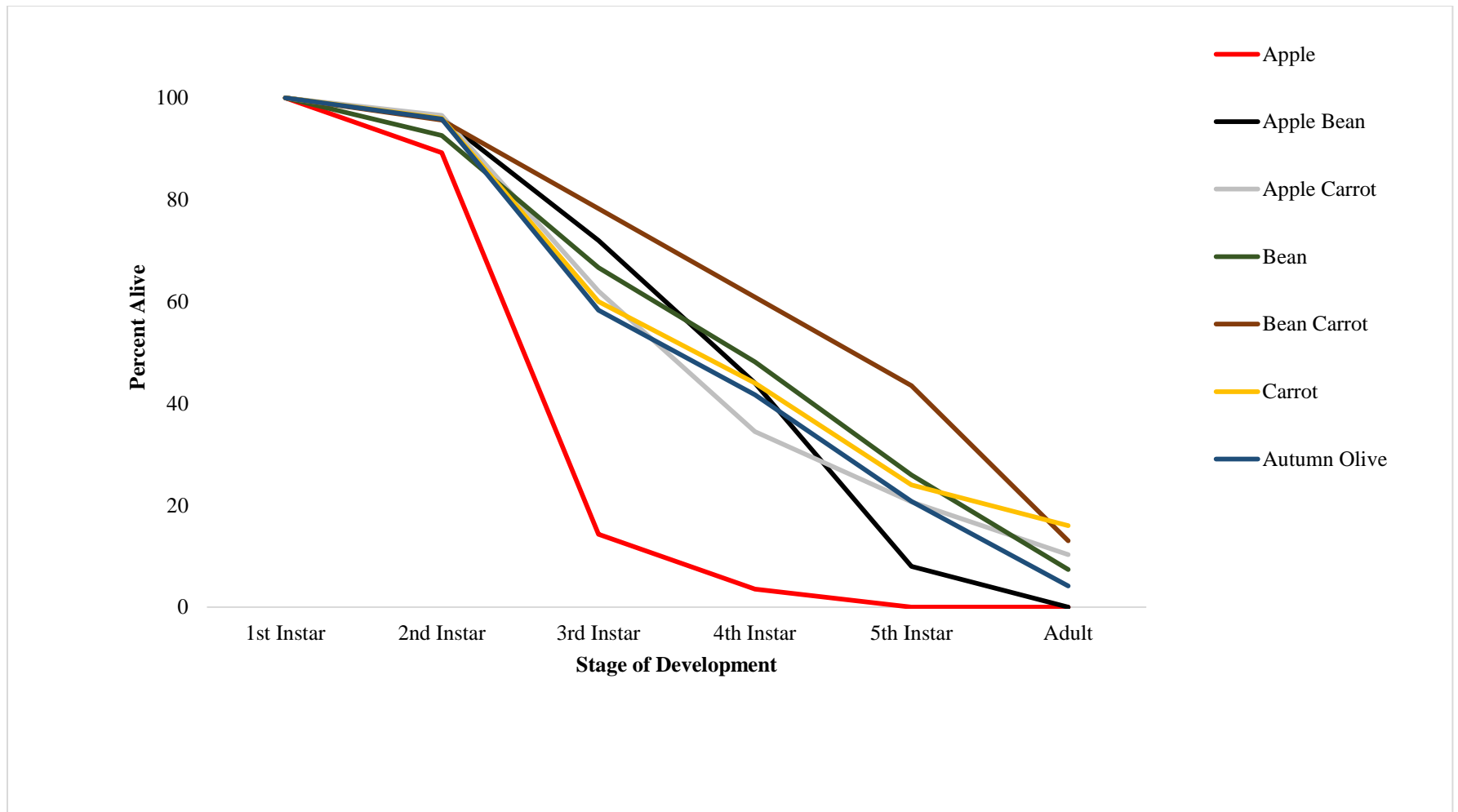


Figure 9: Percentage of brown marmorated stink bugs alive at each stage of development in 2014 diet trail.

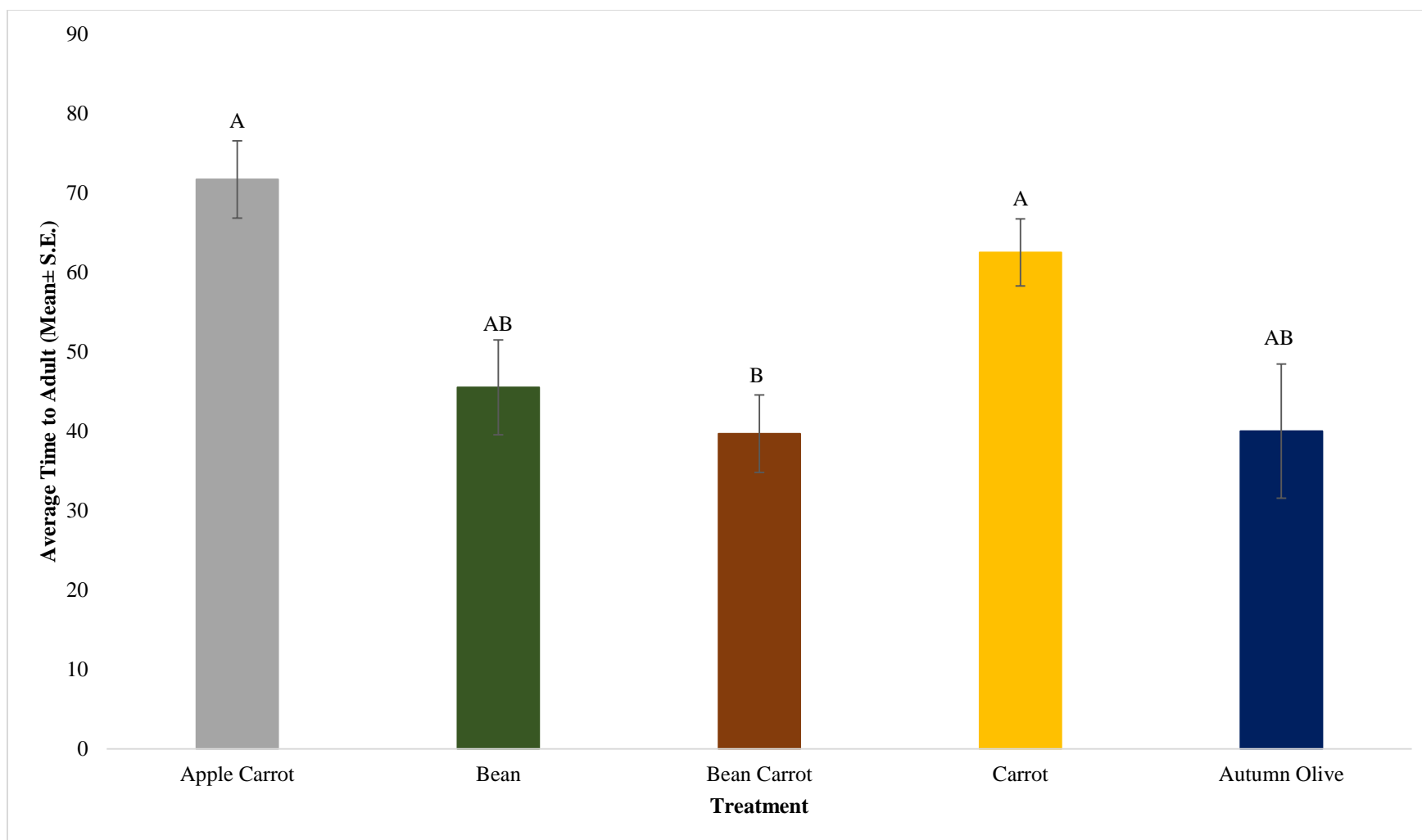


Figure 10: Influence of treatments on brown marmorated stink bugs time to adulthood in diet trial 2014. Bars with the same letter are not significantly different according to Tukey's test ($\alpha=0.05$).

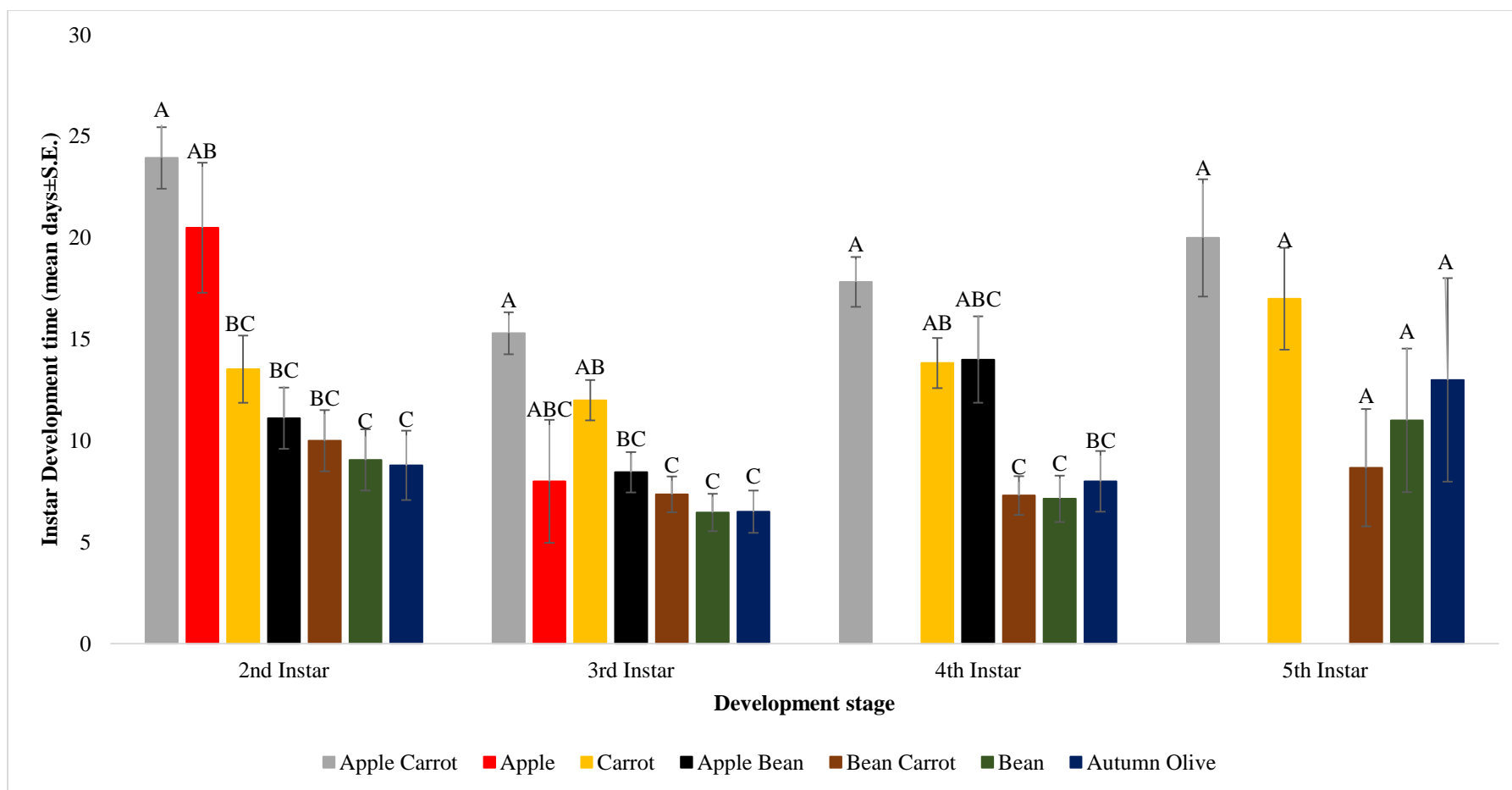


Figure 11: The average time spent at each instar for the brown marmorated stink bug in the 2014 diet trial. Bars with the same letter over them are not significantly different within that grouping according to Tukey's test (alpha=0.05).

2015:

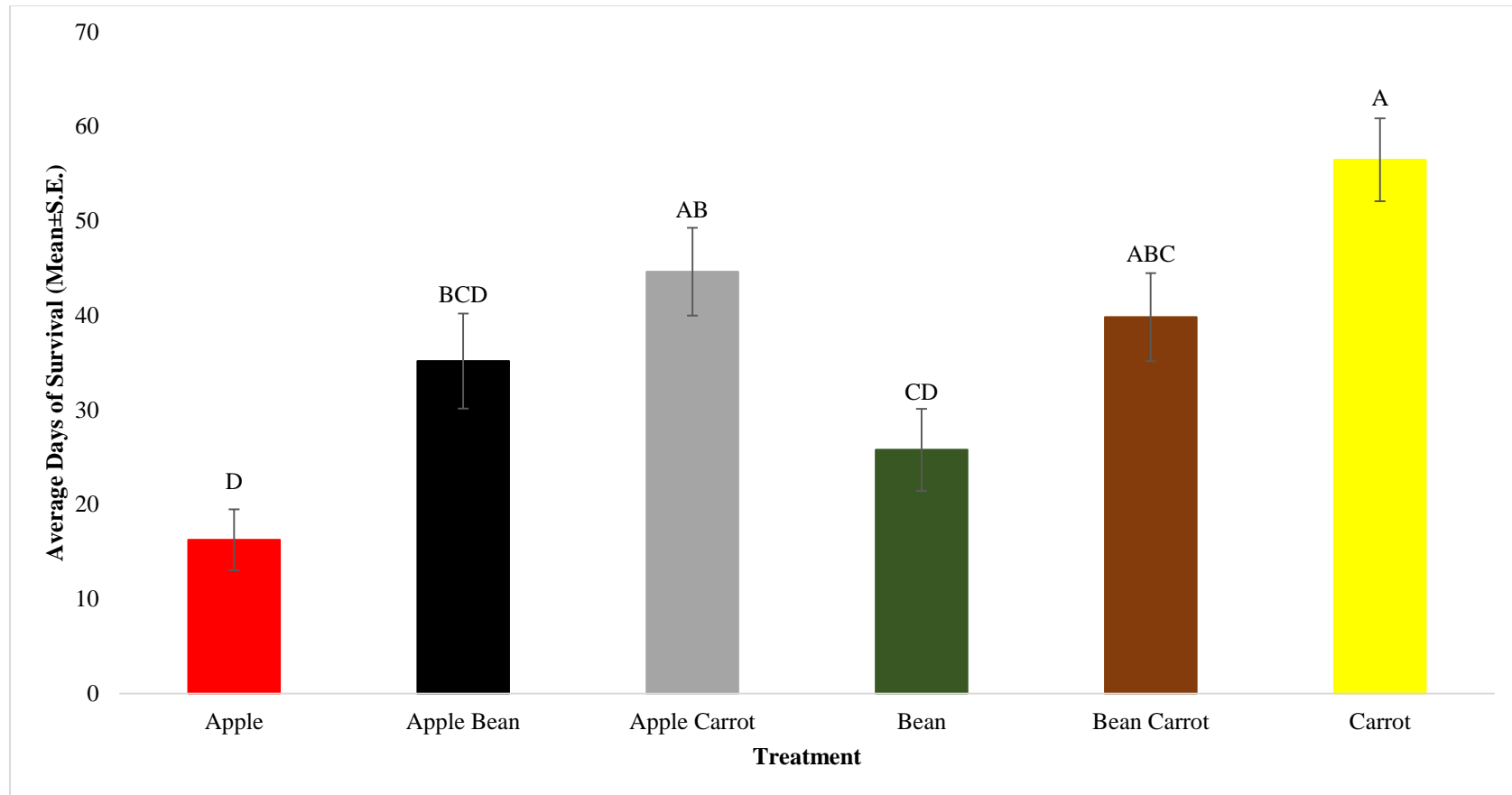


Figure 12: Influence of diet treatment on brown marmorated stink bug survival in 2015 diet trial. Bars with the same letter are not significantly different according to Tukey's test ($\alpha=0.05$).

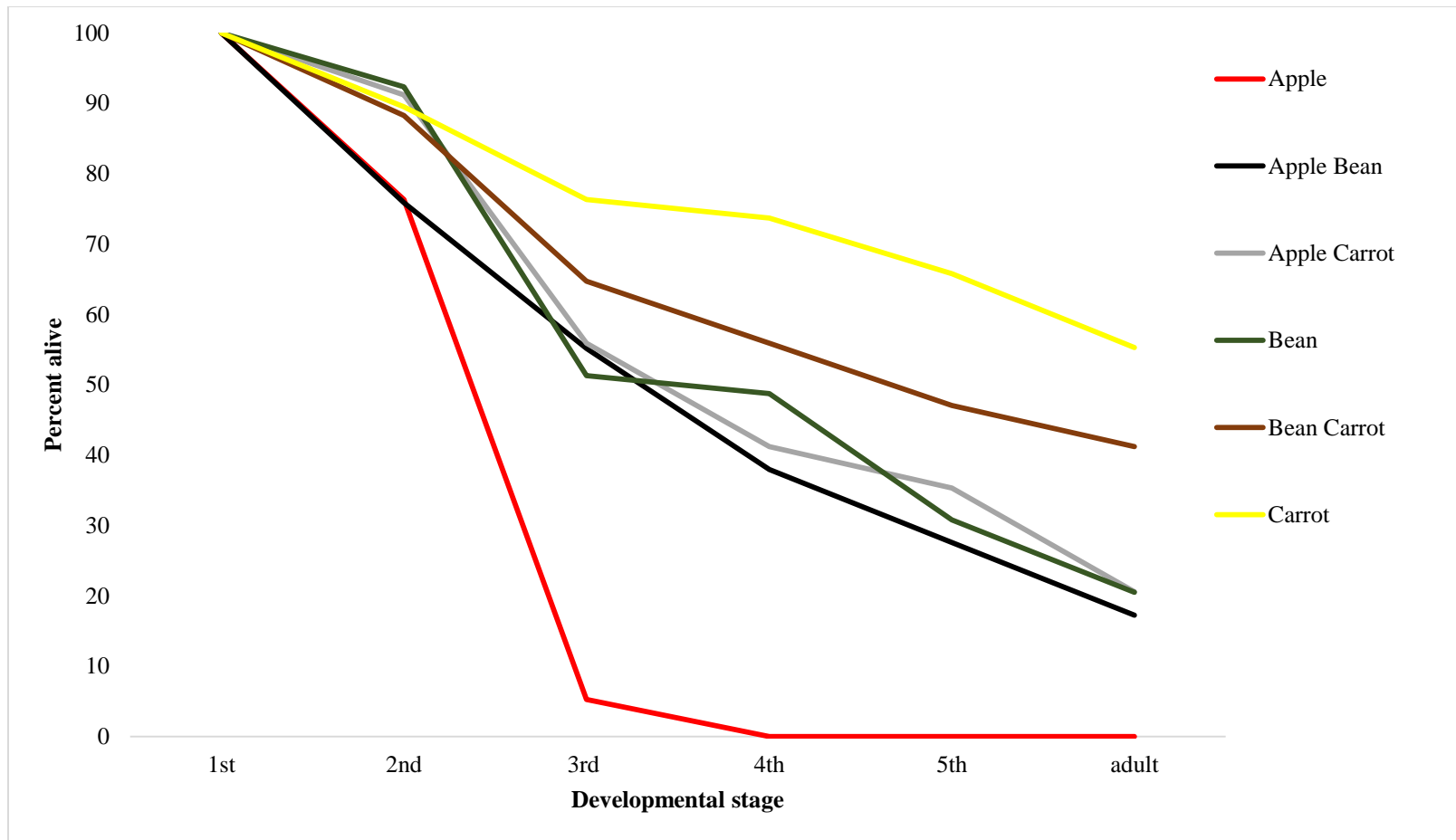


Figure 13: Percentage of brown marmorated stink bugs alive at each stage of development in 2015 diet trail.

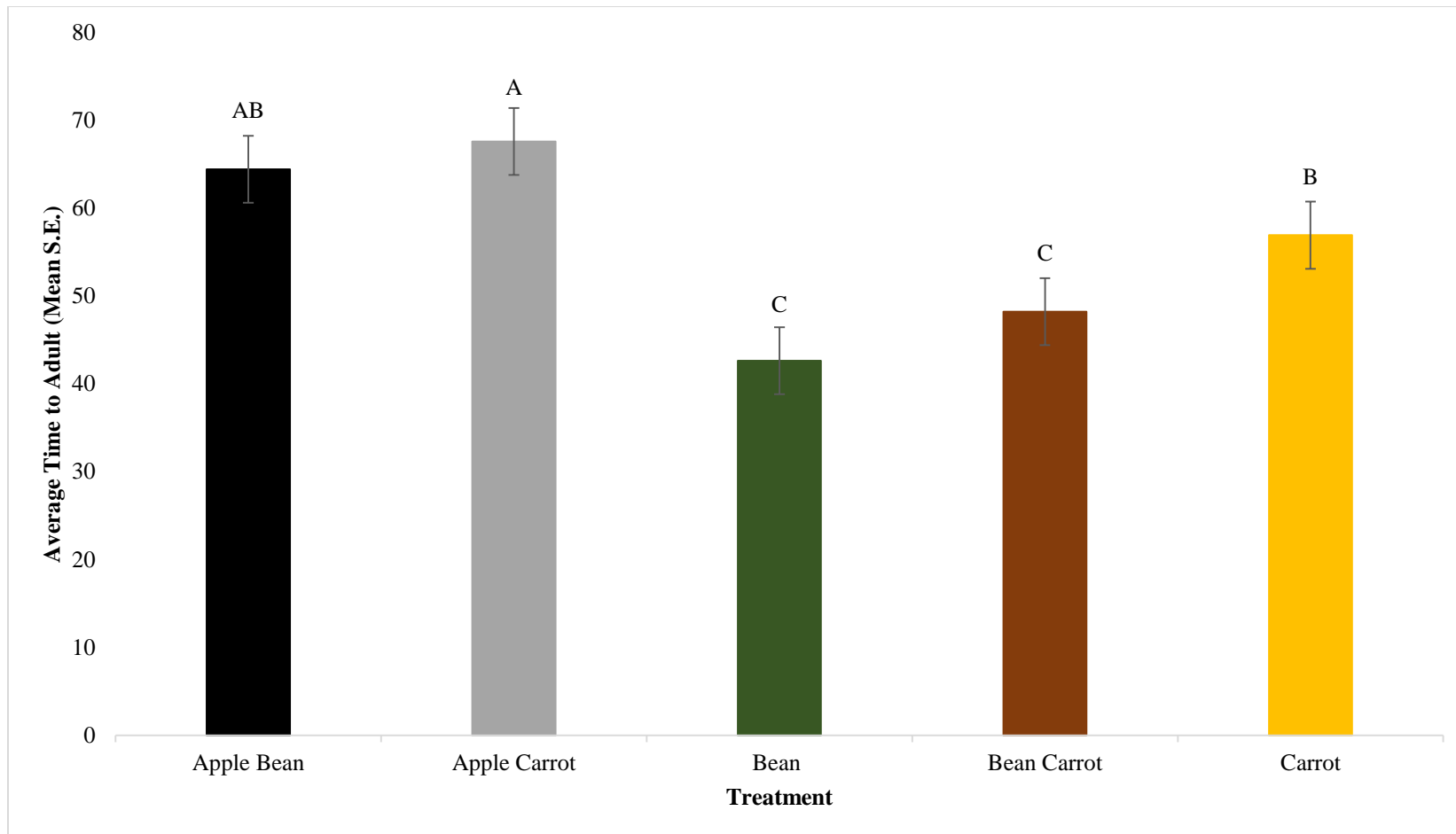


Figure 14: Influence of treatments on brown marmorated stink bugs time to adulthood in diet trial 2015. Bars with the same letter are not significantly different according to Tukey's test ($\alpha=0.05$).

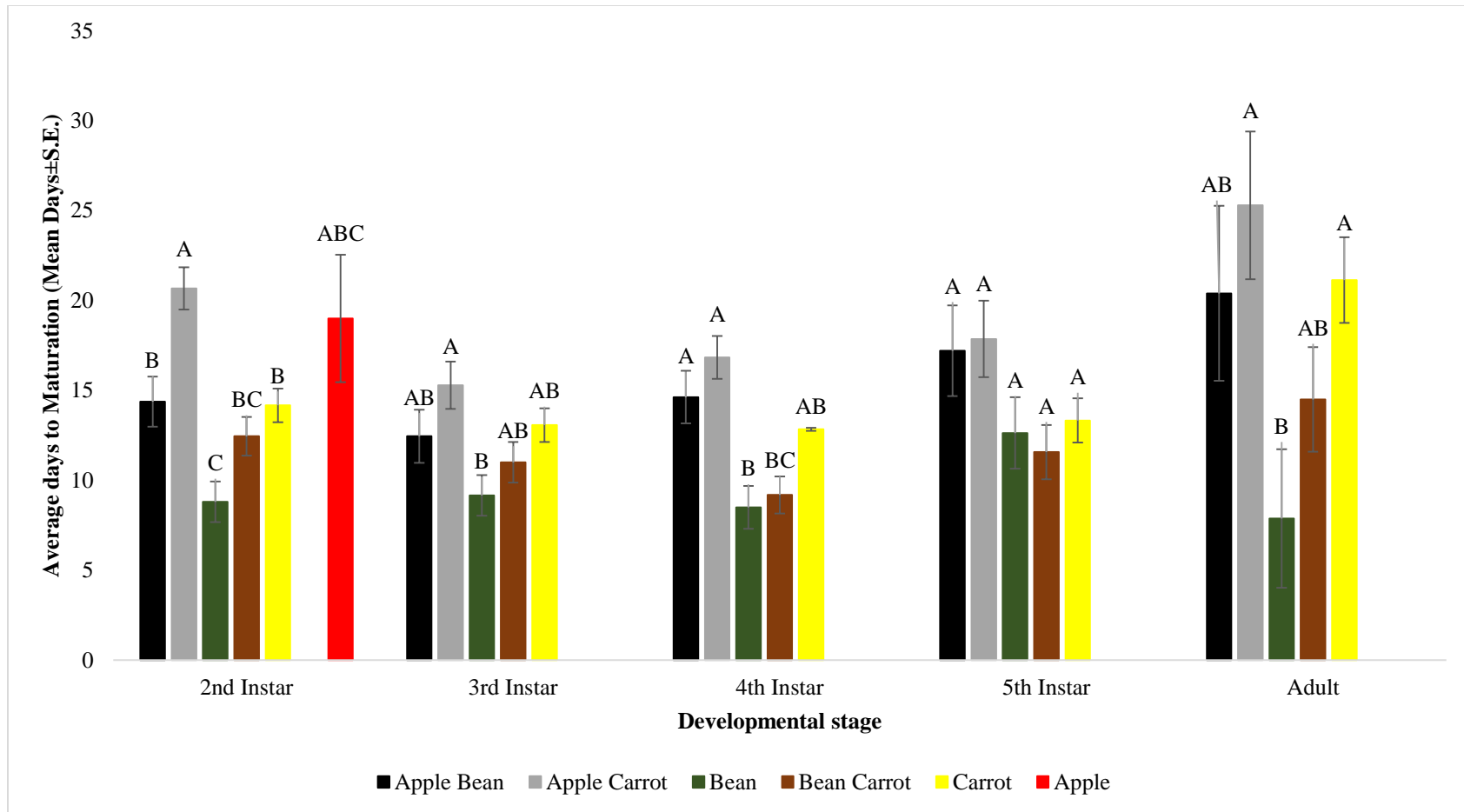


Figure 15: The average time spent at each instar for the brown marmorated stink bug in the 2015 diet trial. Bars with the same letter over them are not significantly different within that grouping according to Tukey's test ($\alpha=0.05$). Note: developmental stage adult is time from becoming an adult until death.

Table 5: Total number and weight of adults produced from 2014 and 2015 diet trials.

| Diet | 2014 | | 2015 | |
|--------------|--------------------|---------------|-------------|---------------|
| | Male | Female | Male | Female |
| Apple | 0 ¹ | 0 | 0 | 0 |
| | - | - | - | - |
| Apple Bean | 0 | 0 | 2 | 3 |
| | - | - | 0.067 | 0.119 |
| Apple Carrot | 1 | 1 | 7 | 0 |
| | 0.093 ² | 0.097 | 0.071 | - |
| Bean | 2 | 0 | 6 | 2 |
| | 0.081 | - | 0.090 | 0.112 |
| Bean Carrot | 1 | 1 | 13 | 1 |
| | 0.102 | 0.087 | 0.077 | 0.131 |
| Carrot | 1 | 3 | 15 | 5 |
| | 0.071 | 0.088 | 0.079 | 0.104 |
| Autumn Olive | 0 | 0 | - | - |
| | - | - | - | - |

1: Total number.

2: Average weight (g).

Table 6: Log rank test of survival functions for diet treatments.

| Year | X² | DF | P |
|-------------|----------------------|-----------|----------|
| 2014 | 19.94 | 5 | 0.0013 |
| 2015 | 53.78 | 5 | <0.0001 |

Table 7: Bonferroni adjustment for multiple comparisons of log rank test on diet treatments for 2014

| Treatment | Treatment | Chi-square | Adjusted p-value |
|------------------|------------------|-------------------|-------------------------|
| Apple | Apple Bean | 1.4415 | 1.0000 |
| Apple | Apple Carrot | 3.4011 | 0.9773 |
| Apple | Bean | 3.2139 | 1.0000 |
| Apple | Bean Carrot | 0.7384 | 1.0000 |
| Apple | Carrot | 0.2203 | 1.0000 |
| Apple Bean | Apple Carrot | 10.2297 | 0.0207 |
| Apple Bean | Bean | 0.3967 | 1.0000 |
| Apple Bean | Bean Carrot | 0.1439 | 1.0000 |
| Apple Bean | Carrot | 3.0878 | 1.0000 |
| Apple Carrot | Bean | 14.0613 | 0.0027 |
| Apple Carrot | Bean Carrot | 8.2889 | 0.0598 |
| Apple Carrot | Carrot | 1.9585 | 1.0000 |
| Bean | Bean Carrot | 1.0424 | 1.0000 |
| Bean | Carrot | 5.4833 | 0.2880 |
| Bean Carrot | Carrot | 1.9894 | 1.0000 |

Table 8: Bonferroni adjustment for multiple comparisons of log rank test on diet treatments for 2015

| Treatment | Treatment | Chi-square | Adjusted p-value |
|------------------|------------------|-------------------|-------------------------|
| Apple | Apple Bean | 9.8296 | 0.0259 |
| Apple | Apple Carrot | 20.0682 | 0.0001 |
| Apple | Bean | 1.0630 | 1.0000 |
| Apple | Bean Carrot | 8.2833 | 0.0600 |
| Apple | Carrot | 31.6436 | <0.0001 |
| Apple Bean | Apple Carrot | 2.1364 | 1.0000 |
| Apple Bean | Bean | 3.8081 | 0.7651 |
| Apple Bean | Bean Carrot | 0.00723 | 1.0000 |
| Apple Bean | Carrot | 6.9868 | 0.1232 |
| Apple Carrot | Bean | 11.0005 | 0.0137 |
| Apple Carrot | Bean Carrot | 2.2718 | 1.0000 |
| Apple Carrot | Carrot | 1.3384 | 1.0000 |
| Bean | Bean Carrot | 3.0998 | 1.0000 |
| Bean | Carrot | 19.6749 | 0.0001 |
| Bean Carrot | Carrot | 6.9952 | 0.1226 |

Discussion:

Although apples are a prime source of food in the wild, this study showed that when apples were the only food source no individual made it past 4th instar. This effect on survival and molting to the adult stage can be explained by the lack of sterols in apples. Insects, unlike humans, cannot create sterols like cholesterol, instead they must get them in the form of phytosterols from plants. In order for insects to molt they need ecdysone steroid, and in order to create this steroid sterols are required. It is known that sterol levels in apples decrease over time when in cold storage. As stated previously, the apples used in this experiment had been in cold storage to retain freshness. Table 9 shows the amount of phytosterols that fresh produce has. Apples start out with the least amount of phytosterols and then they begin to decrease the longer they are in cold storage after harvest.

Table 9: Average amount of phytosterols (mgs/100 g serving) in the three main foods used in the diet trials

| Food | Phytosterols (mgs/100g serving) |
|-------------|---------------------------------|
| Carrots | 94 |
| Green Beans | 50 |
| Apple | 46 |

(Ostlund 2002)

Although BMSBs given a carrot diet had the longest average survival time, they also on average took the longest time to progress from instar to instar, and in turn to reach adulthood. BMSBs on carrots also spent the most time as adults, meaning if they had a chance to mate they would most likely produce the most egg masses. On the opposite end of the spectrum, BMSBs on a beans and autumn olive diet had the shortest average life span, but also the shortest average time between molts and to adulthood.

Overall survival time was greatest with a carrot diet, but it was not significantly different from carrot apple, apple, or bean carrot. BMSBs on diets containing carrot tended to survive

longer while diets with beans in them (besides bean carrot) survived on average for shorter period of time. This idea of carrots adding to the longevity of life was first written about by Funayana (2006), where it was shown that adding carrot to a soybean-pod based diet increased lifespan and fecundity. Also, as seen in Table 9, carrots have the most phytosterols out of the three food items.

As seen in 2014, the trend of BMSBs on treatments containing carrots had significantly longer survival periods, compared to treatments with beans and other foods. In 2015, BMSBs on the treatment of apple did not produce as many nymphs surviving at 2nd and 3rd instar for long periods of time, as was seen in 2014. The increased replications in 2015 decreased the effects caused by a few outliers in 2014.

Also, similar to 2014, in the 2015 trials BMSBs that fed on apples took the longest amount of time between molts, followed by treatments with carrots, and then treatments with beans having the shortest time between molts. It is worth noting that in 2015 the time spent alive after becoming an adult was significantly greater for treatments with carrots in them. It is also worth noting that in 2015 two individual females laid eggs (unfertilized) in their cups. One female on the apple bean treatment laid two egg masses both containing 27 eggs 12 days apart. One female from the bean carrot treatment laid an egg mass of 28 eggs.

Overall, there is no clear winner between multiple foods treatments versus having one food treatment. In this study examples can be found where BMSBs on a single diet outperformed a BMSB on a mixed diet or the other way around. As mentioned previously, past studies suggested that adding carrots to a soybean based diet for BMSBs would help increase fecundity and survival of future generations of BMSBs (Funayama, 2006). This is very logical because an increase in lifespan would allow for more time to produce a greater number of egg

masses. Therefore it can be concluded that a mixed diet is most likely the most beneficial for survivability. Beans and carrots are the best choice because beans increase the rate at which each instar stage is reached and carrots help to increase life span.

There are myriad opportunities for future research, such as investigating the fecundity of adults that have been reared solely on these aforementioned diets. What happens to these cohorts once they get to second and third generations? Another possible avenue to investigate is why are more adult males surviving than adult females? Females in general are larger and heavier, so do they require more food than what was given in order to have greater chance of sustaining their larger size? There are many questions that are still unanswered that could lead to greatly improving the health of lab-raised colonies of BMSBs. The problem of BMSB damage to agricultural crops is not soon to be solved, so the need for lab-raised colonies to support research will persist through the foreseeable future.

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